

Preliminary Amendment  
Applicant(s): SRIENC et al.  
Serial No. 10/090,965  
Filed: March 4, 2002  
For: *PRODUCTION OF POLYHYDROXYALKANOATES*

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### Amendments to the Claims

This listing of claims replaces all prior versions, and listings, of claims in the above-identified application:

1. (original) A method for the production of a polyhydroxyalkanoate (PHA) comprising:  
providing a transgenic yeast cell comprising a first nucleic acid fragment comprising a heterologous nucleotide sequence encoding a PHA polymerase and at least one second nucleic acid fragment comprising a heterologous nucleotide sequence selected from the group consisting of a heterologous nucleotide sequence encoding an acetoacetyl-CoA reductase and a heterologous nucleotide sequence encoding a  $\beta$ -ketothiolase;  
culturing the transgenic yeast cell under anaerobic conditions to cause the production of PHA; and  
isolating the PHA from the yeast cell.
2. (original) The method of claim 1 wherein the first and second nucleic acid fragments constitute a single nucleic acid fragment.
3. (original) The method of claim 2 wherein the single nucleic acid fragment comprises a divergent promoter operably linked to two of the heterologous nucleotide sequences.
4. (original) The method of claim 1 wherein the yeast cell comprises a second nucleic acid fragment comprising a heterologous nucleotide sequence encoding an acetoacetyl-CoA reductase and a third nucleic acid fragment comprising a nucleotide sequence encoding a  $\beta$ -ketothiolase.
5. (original) The method of claim 4 wherein at least two of the first, second and third nucleic acid fragments constitute a single nucleic acid fragment.
6. (original) The method of claim 5 wherein the single nucleic acid fragment comprises a divergent promoter operably linked to two of the heterologous nucleotide sequences.

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7. (original) The method of claim 1 wherein at least one nucleic acid fragment is integrated into the genome of the yeast cell.
8. (original) The method of claim 1 further comprising introducing at least one nucleic acid fragment into the yeast cell to yield the transgenic yeast cell.
9. (original) The method of claim 1 wherein the yeast cell is a cell from the genus *Saccharomyces*.
10. (original) The method of claim 1 wherein the yeast cell is an *S. cerevisiae* cell.
11. (original) The method of claim 1 wherein the yeast cell is a cell from the genus *Kluyveromyces*.
12. (original) The method of claim 1 wherein the PHA polymerase comprises a PHA<sub>SCL</sub> polymerase.
13. (original) The method of claim 1 wherein the PHA polymerase comprises a PHA<sub>MCL</sub> polymerase.
14. (original) A method for the production of a polyhydroxyalkanoate (PHA) comprising:  
providing a transgenic yeast cell comprising a first nucleic acid fragment comprising heterologous nucleotide sequence encoding a PHA polymerase; at least one second nucleic acid fragment comprising a heterologous nucleotide sequence selected from the group consisting of a heterologous nucleotide sequence encoding an acetoacetyl-CoA reductase and a heterologous nucleotide sequence encoding a  $\beta$ -ketothiolase; and a third nucleic acid fragment comprising a heterologous nucleotide sequence encoding a citrate lyase;  
culturing the transgenic yeast cell to cause the production of PHA; and

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isolating the PHA from the yeast cell.

15. (original) The method of claim 14 wherein the yeast cell comprises a heterologous nucleotide sequence encoding an acetoacetyl-CoA reductase and a heterologous nucleotide sequence encoding a  $\beta$ -ketothiolase.
16. (original) The method of claim 14 wherein at least two of the first, second and third nucleic acid fragments constitute a single nucleic acid fragment.
17. (original) The method of claim 16 wherein the single nucleic acid fragment comprises a divergent promoter operably linked to two of the heterologous nucleotide sequences.
18. (original) The method of claim 14 wherein the yeast cell is a *S. cerevisiae* cell.
19. (original) The method of claim 14 wherein the PHA polymerase comprises a PHA<sub>SCL</sub> polymerase.
20. (original) The method of claim 14 wherein the PHA polymerase comprises a PHA<sub>MCL</sub> polymerase.
21. (original) A method for the production of a polyhydroxyalkanoate (PHA) comprising:  
providing a transgenic yeast cell having transhydrogenase activity, the transgenic yeast cell comprising a first nucleic acid fragment comprising heterologous nucleic acid encoding a PHA polymerase and at least one second nucleic acid fragment comprising a heterologous nucleotide sequence selected from the group consisting of a heterologous nucleotide sequence encoding an acetoacetyl-CoA reductase and a heterologous nucleotide sequence encoding a  $\beta$ -ketothiolase;  
culturing the transgenic yeast cell under conditions to cause the production of PHA; and  
isolating the PHA from the yeast cell.

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22. (original) The method of claim 21 wherein the yeast cell is a *S. cerevisiae* cell.
23. (original) The method of claim 21 wherein overexpression of a glutamate dehydrogenase enzyme in the yeast cell produces the transhydrogenase activity.
24. (original) The method of claim 21 wherein overexpression of a malic enzyme in the yeast cell produces the transhydrogenase activity.
25. (original) The method of claim 21 wherein the PHA polymerase comprises a PHA<sub>SCL</sub> polymerase.
26. (original) The method of claim 21 wherein the PHA polymerase comprises a PHA<sub>MCL</sub> polymerase.
27. (original) A method for the production of a polyhydroxyalkanoate (PHA) comprising:  
providing a transgenic yeast cell comprising a first nucleic acid fragment comprising heterologous nucleotide sequence encoding a PHA<sub>MCL</sub> polymerase and at least one second nucleic acid fragment comprising a heterologous nucleotide sequence selected from the group consisting of a heterologous nucleotide sequence encoding an acetoacetyl-CoA reductase and a heterologous nucleotide sequence encoding a  $\beta$ -ketothiolase;  
culturing the transgenic yeast cell under aerobic conditions to cause the production of PHA; and  
isolating the PHA from the yeast cell.
28. (original) The method of claim 27 wherein at least one nucleic acid fragment comprises a constitutive promoter linked to at least one heterologous nucleotide sequence.

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29. (original) The method of claim 27 wherein the yeast cell comprises a second nucleic acid fragment comprising a heterologous nucleotide sequence encoding an acetoacetyl-CoA reductase and a third nucleic acid fragment comprising a nucleotide sequence encoding a  $\beta$ -ketothiolase.
30. (original) The method of claim 29 wherein at least two of the first, second and third nucleic acid fragments constitute a single nucleic acid fragment.
31. (original) The method of claim 30 wherein the single nucleic acid fragment comprises a constitutive divergent promoter operably linked to each of the heterologous nucleic acid sequences.
32. (original) The method of claim 27 wherein at least one nucleic acid fragment is integrated into the genome of the yeast cell.
33. (original) The method of claim 27 wherein the yeast cell is an *S. cerevisiae* cell
34. (original) A method for production of a polyhydroxyalkanoate (PHA) and ethanol comprising:  
culturing a transgenic yeast cell comprising a first nucleic acid fragment comprising a heterologous nucleotide sequence encoding a PHA polymerase and at least one second nucleic acid fragment comprising a heterologous nucleotide sequence selected from the group consisting of a heterologous nucleotide sequence encoding an acetoacetyl-CoA reductase and a heterologous nucleotide sequence encoding a  $\beta$ -ketothiolase under conditions to cause the production of ethanol;  
isolating the ethanol from the cell culture;  
culturing the yeast cell under conditions to cause the production of PHA; and  
isolating the PHA from the yeast cell.

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35. (original) The method of claim 34 wherein culturing the yeast cell under conditions to cause the production of PHA comprises supplying the yeast cell with a feed comprising at least one component selected from the group consisting of acetate, propionate and valerate.

36. (original) The method of claim 34 wherein first and second nucleic acid fragments constitute a single nucleic acid fragment.

37. (original) The method of claim 36 wherein the single nucleic acid fragment comprises a divergent promoter operably linked to two of the heterologous nucleotide sequences.

38. (original) The method of claim 34 wherein the yeast cell comprises a second nucleic acid fragment comprising a heterologous nucleotide sequence encoding an acetoacetyl-CoA reductase and a third nucleic acid fragment comprising a nucleotide sequence encoding a  $\beta$ -ketothiolase.

39. (original) The method of claim 38 wherein at least two of the first, second and third nucleic acid fragments constitute a single nucleic acid fragment.

40. (original) The method of claim 39 wherein the single nucleic acid fragment comprises a divergent promoter operably linked to two of the heterologous nucleotide sequences.

41. (original) The method of claim 34 wherein at least one nucleic acid fragment is integrated into the genome of the yeast cell.

42. (original) The method of claim 34 further comprising introducing at least one nucleic acid fragment into the yeast cell to yield the transgenic yeast cell.

43. (original) The method of claim 34 wherein the yeast cell is a cell from the genus *Saccharomyces*.

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44. (original) The method of claim 34 wherein the yeast cell is an *S. cerevisiae* cell
45. (original) The method of claim 34 wherein the yeast cell is a cell from the genus *Kluyveromyces*.
46. (original) The method of claim 34 wherein the PHA polymerase comprises a PHA<sub>SCL</sub> polymerase.
47. (original) The method of claim 34 wherein the PHA polymerase comprises a PHA<sub>MCL</sub> polymerase.
48. (original) The method of claim 34 wherein the yeast cell further comprises a heterologous nucleotide sequence encoding a citrate lyase.
49. (original) The method of claim 34 wherein the yeast cell has transhydrogenase activity.
50. (original) The method of claim 34 wherein the yeast cell is cultured in a first fermentation chamber under conditions to cause the production of ethanol; and wherein the yeast cell is cultured in a second fermentation chamber under conditions to cause the production of PHA.
51. (original) A method for production of a polyhydroxyalkanoate (PHA) and lactic acid comprising:  
culturing a transgenic yeast cell comprising a first nucleic acid fragment comprising a heterologous nucleotide sequence encoding a PHA polymerase and at least one second nucleic acid fragment comprising a heterologous nucleotide sequence selected from the group consisting of a heterologous nucleotide sequence encoding an acetoacetyl-CoA reductase and a heterologous nucleotide sequence encoding a  $\beta$ -ketothiolase under conditions to cause the production of lactic acid;

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isolating the lactic acid from the cell culture;  
culturing the yeast cell under conditions to cause the production of PHA; and  
isolating the PHA from the yeast cell.

52. (original) The method of claim 51 wherein culturing the yeast cell under conditions to cause the production of PHA comprises supplying the yeast cell with a feed comprising at least one component selected from the group consisting of acetate, propionate and valerate.

53. (original) The method of claim 51 wherein the yeast cell comprises a heterologous nucleotide sequence encoding an acetoacetyl-CoA reductase and a heterologous nucleotide sequence encoding a  $\beta$ -ketothiolase.

54. (original) The method of claim 51 wherein first and second nucleic acid fragments constitute a single nucleic acid fragment.

55. (original) The method of claim 54 wherein the single nucleic acid fragment comprises a divergent promoter operably linked to two of the heterologous nucleotide sequences.

56. (original) The method of claim 51 wherein the yeast cell is a *S. cerevisiae* cell.

57. (original) The method of claim 51 wherein the PHA polymerase comprises a PHA<sub>SCL</sub> polymerase.

58. (original) The method of claim 51 wherein the PHA polymerase comprises a PHA<sub>MCL</sub> polymerase.

59. (original) The method of claim 51 wherein the yeast cell further comprises a heterologous nucleotide sequence encoding a citrate lyase.



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60. (original) The method of claim 51 wherein the yeast cell has transhydrogenase activity.
61. (original) The method of claim 51 wherein the yeast cell is cultured in a first fermentation chamber under conditions to cause the production of ethanol; and wherein the yeast cell is cultured in a second fermentation chamber under conditions to cause the production of PHA.
62. (original) A method for the production of a polyhydroxyalkanoate (PHA) comprising:  
providing a transgenic bacterial cell comprising a first nucleic acid fragment comprising a heterologous nucleotide sequence encoding a PHA polymerase and at least one second nucleic acid fragment comprising a heterologous nucleotide sequence selected from the group consisting of a heterologous nucleotide sequence encoding an acetoacetyl-CoA reductase and a heterologous nucleotide sequence encoding a  $\beta$ -ketothiolase;  
culturing the bacterial cell under anaerobic conditions to cause the production of PHA;  
and  
isolating the PHA from the bacterial cell.
63. (original) The method of claim 62 wherein the bacterial cell comprises a second nucleic acid fragment comprising a heterologous nucleotide sequence encoding an acetoacetyl-CoA reductase and a third nucleic acid fragment comprising a nucleotide sequence encoding a  $\beta$ -ketothiolase.
64. (original) The method of claim 63 wherein at least two of the first, second and third nucleic acid fragments constitute a single nucleic acid fragment.
65. (original) The method of claim 62 wherein the PHA polymerase comprises a PHA<sub>MCL</sub> polymerase.

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66. (original) The method of claim 62 wherein the PHA polymerase comprises a PHA<sub>SCL</sub> polymerase.

67. (original) The method of claim 62 wherein the bacterial cell is from a genus selected from the group consisting of *Escherichia*, *Zooglea* and *Lactobacillus*.

68. (original) A method for production of a polyhydroxyalkanoate (PHA) and ethanol in a transgenic bacterial cell comprising:

providing a transgenic bacterial cell comprising a first nucleic acid fragment comprising a heterologous nucleotide sequence encoding a PHA polymerase and at least one second nucleic acid fragment comprising a heterologous nucleotide sequence selected from the group consisting of a heterologous nucleotide sequence encoding an acetoacetyl-CoA reductase and a heterologous nucleotide sequence encoding a  $\beta$ -ketothiolase;

culturing the bacterial cell under conditions to cause the production of ethanol;

isolating the ethanol from the cell culture;

culturing the bacterial cell under conditions to cause the production of PHA; and

isolating the PHA from the bacterial cell.

69. (original) The method of claim 68 wherein the bacterial cell comprises a second nucleic acid fragment comprising a heterologous nucleotide sequence encoding an acetoacetyl-CoA reductase and a third nucleic acid fragment comprising a nucleotide sequence encoding a  $\beta$ -ketothiolase.

70. (original) The method of claim 69 wherein at least two of the first, second and third nucleic acid fragments constitute a single nucleic acid fragment.

71. (original) The method of claim 68 wherein culturing the transgenic bacterial cell under conditions to cause the production of PHA comprises supplying the bacterial cell with a feed

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comprising at least one component selected from the group consisting of acetate, propionate and valerate.

72. (original) The method of claim 68 wherein the bacterial cell is from a genus selected from the group consisting of *Escherichia*, *Zooglea* and *Lactobacillus*.

73. (original) A method for the production of a polyhydroxyalkanoate (PHA) comprising:  
providing a transgenic *E. coli* cell comprising a first nucleic acid fragment comprising a heterologous nucleotide sequence encoding a PHA polymerase and at least one second nucleic acid fragment comprising a heterologous nucleotide sequence selected from the group consisting of a heterologous nucleotide sequence encoding an acetoacetyl-CoA reductase and a heterologous nucleotide sequence encoding a  $\beta$ -ketothiolase;  
culturing the *E. coli* cell under anaerobic conditions to cause the production of PHA; and  
isolating the PHA from the *E. coli* cell.

74. (original) The method of claim 73 wherein the *E. coli* cell comprises a second nucleic acid fragment comprising a heterologous nucleotide sequence encoding an acetoacetyl-CoA reductase and a third nucleic acid fragment comprising a heterologous nucleotide sequence encoding a  $\beta$ -ketothiolase.

75. (original) The method of claim 74 wherein at least two of the first, second and third nucleic acid fragments constitute a single nucleic acid fragment.

76. (original) The method of claim 73 wherein the PHA polymerase comprises a PHA<sub>MCL</sub> polymerase.

77. (original) The method of claim 73 wherein the PHA polymerase comprises a PHA<sub>SSL</sub> polymerase.

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78. (original) A method for the production of a polyhydroxyalkanoate (PHA) comprising:  
providing a transgenic *E. coli* cell comprising a first nucleic acid fragment comprising a heterologous nucleotide sequence encoding a PHA polymerase; a second nucleic acid fragment comprising a heterologous nucleotide sequence encoding an acetoacetyl-CoA reductase; and a third nucleic acid fragment comprising a heterologous nucleotide sequence encoding a  $\beta$ -ketothiolase;  
culturing the *E. coli* cell under anaerobic conditions to cause the production of PHA; and  
isolating the PHA from the *E. coli* cell.
79. (original) The method of claim 78 wherein at least two of the first, second and third nucleic acid fragments constitute a single nucleic acid fragment.
80. (original) The method of claim 78 wherein the PHA polymerase comprises a PHA<sub>MCL</sub> polymerase.
81. (original) The method of claim 78 wherein the PHA polymerase comprises a PHA<sub>SCI</sub> polymerase.
82. (original) A method for production of a polyhydroxyalkanoate (PHA) and ethanol in a transgenic *E. coli* cell comprising:  
providing a transgenic *E. coli* cell comprising a first nucleic acid fragment comprising a heterologous nucleotide sequence encoding a PHA polymerase and at least one second nucleic acid fragment comprising a heterologous nucleotide sequence selected from the group consisting of a heterologous nucleotide sequence encoding an acetoacetyl-CoA reductase and a heterologous nucleotide sequence encoding a  $\beta$ -ketothiolase;  
culturing the *E. coli* cell under conditions to cause the production of ethanol;  
isolating the ethanol from the cell culture;  
culturing the *E. coli* cell under conditions to cause the production of PHA; and  
isolating the PHA from the *E. coli* cell.

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83. (original) The method of claim 82 wherein culturing the transgenic bacterial cell under conditions to cause the production of PHA comprises supplying the bacterial cell with a feed comprising at least one component selected from the group consisting of acetate, propionate and valerate.

84. (original) A method for production of a polyhydroxyalkanoate (PHA) and lactic acid comprising:

providing a transgenic bacterial cell comprising a first nucleic acid fragment comprising a heterologous nucleotide sequence encoding a PHA polymerase and at least one second nucleic acid fragment comprising a heterologous nucleotide sequence selected from the group consisting of a heterologous nucleotide sequence encoding an acetoacetyl-CoA reductase and a heterologous nucleotide sequence encoding a  $\beta$ -ketothiolase;

culturing the bacterial cell under conditions to cause the production of lactic acid;  
isolating the lactic acid from the cell culture;  
culturing the bacterial cell under conditions to cause the production of PHA; and  
isolating the PHA from the bacterial cell.

85. (original) The method of claim 84 wherein culturing the bacterial cell under conditions to cause the production of PHA comprises supplying the bacterial cell with a feed comprising at least one component selected from the group consisting of acetate, propionate and valerate.

86. (original) The method of claim 84 wherein the transgenic bacterial cell is from a genus selected from the group consisting of *Escherichia*, *Zooglea* and *Lactobacillus*.

87. (original) A transgenic yeast cell comprising a heterologous PHA<sub>MCL</sub> polymerase and at least one enzyme selected from a heterologous acetoacetyl-CoA reductase and a heterologous  $\beta$ -ketothiolase.

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88. (original) The transgenic yeast cell of claim 87 which is a *S. cerevisiae* cell.
89. (original) A transgenic yeast cell comprising a heterologous PHA polymerase, a heterologous acetoacetyl-CoA reductase, and a heterologous citrate lyase.
90. (original) The transgenic yeast cell of claim 89 further comprising a heterologous  $\beta$ -ketothiolase.
91. (original) The transgenic yeast cell of claim 89 wherein the PHA polymerase comprises a PHA<sub>SCL</sub> polymerase.
92. (original) The transgenic yeast cell of claim 89 wherein the PHA polymerase comprises a PHA<sub>MCL</sub> polymerase.
93. (original) The transgenic yeast cell of claim 89 which is a *S. cerevisiae* cell.
94. (new) The method of claim 1 further comprising, prior to culturing the transgenic yeast cell under anaerobic conditions to cause production of PHA, culturing the transgenic yeast cell under conditions to cause the production of ethanol and isolating the ethanol from the cell culture.